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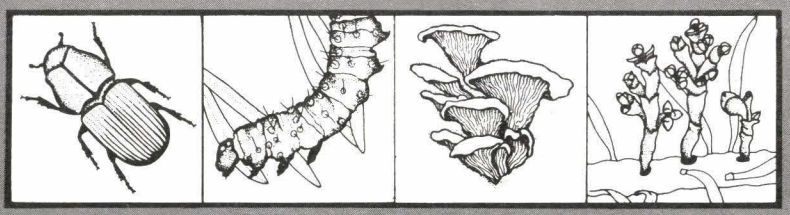

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OCCURRENCE OF *FUSARIUM* ON DOUGLAS-FIR SEED AND CONTAINERIZED SEEDLINGS AT THE PLUM CREEK NURSERY, PABLO, MONTANA

by

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ABSTRACT

Investigations were conducted to determine importance of *Fusarium* as a pathogen on Douglas-fir seed and containerized seedlings at the Plum Creek Nursery during 1985. *Fusarium oxysporum* was occasionally isolated from necrotic lesions on young germinants that lacked foliar symptoms. Low levels of *F. oxysporum* were detected on three of four seedlots sampled; *F. acuminatum* was found on only one seedlot. Treatment effects to reduce levels of *Fusarium* were inconclusive, primarily because of low pathogen levels. Occurrence of *Fusarium* as a pathogen on this crop was rare in 1985.

INTRODUCTION

A new greenhouse facility to produce containerized conifer seedlings for reforestation was recently built by the Plum Creek Lumber Company at Pablo, Montana. Production capacity is almost 2 million seedlings annually. The first crop was started in the spring of 1985, consisting of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Engelmann spruce (*Picea engelmanni* Parry), lodgepole pine (*Pinus contorta* Dougl.), and western larch (*Larix occidentalis* Nutt.). Several container nurseries in the northern Rocky Mountains have experienced problems with damping-off and root diseases caused by *Fusarium*, particularly on Douglas-fir (James 1983a; James 1984b; James 1984c; James 1985a; James 1985c; James 1985d). Most of these disease problems probably result from infected seed. Although losses are usually not severe, in some cases extensive mortality can result (James and Gilligan 1984).

Because of losses from seed-borne *Fusarium* commonly experienced at other nurseries, growers at the Plum Creek Nursery were concerned about possible losses they might have from these pathogens. Therefore, investigations were conducted on the occurrence of *Fusarium* on the roots of young Douglas-fir germinants, older seedlings, and levels of contamination on selected Douglas-fir seedlots.

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MATERIALS AND METHODS

Isolations from Douglas-fir Seedling Roots - Douglas-fir seed was sown about mid-April in styroblock containers on a standard peat-vermiculite growing medium (Forestry Mix® - W. R. Grace Co.) and covered with grit. Shortly after emergence, Banrot® (a combination of 15 percent terrazole and 25 percent Topsin M - produced by Mallinckrodt) and Benlate® (benomyl=methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate - produced by Dupont) were applied as a prevention against damping-off. During thinning (about 3 weeks after sowing) shrunken rust-colored lesions were found on several germlings (figure 1).

Figure 1.--Three-week-old Douglas-fir germling with rust-colored lesions on its main tap root.

Growers were concerned that these lesions may be due to pathogenic fungi, particularly Fusarium. Thirteen germlings with lesions were taken to the laboratory for analysis. The entire taproot of each germling was surface sterilized in 0.5 percent aqueous sodium hypochlorite (10 percent bleach) for 1 minute and rinsed with distilled water. The taproot was then aseptically cut into 13-15 segments and placed on a medium selective for Fusarium (Komada 1975). Plates were incubated for 5-7 days at about 24°C under a 12 hour

diurnal cycle of fluorescent light and darkness. Emerging fungi were transferred to potato dextrose agar (PDA) slants; Fusarium isolates were grown on carnation leaf agar (CLA) to facilitate their identification (Fisher et al. 1982). Standard taxonomic keys (Booth 1971; Gerlach and Nirenberg 1982) were used to identify all Fusarium isolates.

When Douglas-fir seedlings were 3 months old, they were closely examined for possible root disease symptoms. Needle or tip dieback commonly associated with Fusarium infection of older seedlings (James 1984b; James 1984c) was not found. However, many seedlings displayed twisting of their terminal needles. Some of this twisted foliage was also slightly chlorotic. All Douglas-fir seedlots from Montana were affected with this disorder even though they were from different sources; a seedlot obtained from the Potlatch Nursery (Lewiston, Idaho) was much less affected and produced taller, healthier-appearing seedlings. Thirteen seedlings that were smaller and with excessively twisted needles were analyzed for presence of Fusarium on their roots. Roots were surface sterilized and the tips of 15 randomly selected roots were incubated on the selective Fusarium medium as described previously.

Douglas-fir Seed Treatment and Germination - Seeds from four Douglas-fir seedlots from Montana were subjected to five treatments (table 1) after which they were assayed for presence of Fusarium and other fungi. Seeds were incubated on the selective Fusarium medium as described for seedling root isolations. For each seedlot, 25-30 seed were assayed per treatment. Another 84-100 seeds from each seedlot were subjected to three treatments (table 1) and evaluated for germination on moistened filter paper. Germination tests were conducted at about 24°C under a 12-hour diurnal light/darkness regime. Germination was tallied after incubation for 7 and 19 days. At the end of 19 days, all remaining ungerminated seeds were dissected to determine if they were decayed or empty or if their endosperms appeared sound and germinable.

RESULTS

Isolations from Douglas-fir Seedling Roots - Fusarium was recovered from the roots of six of the 13 germlings sampled (table 2). Extent of colonization ranged from almost 7 percent to more than 23 percent of the taproot. The major Fusarium species isolated was F. oxysporum Schlect.; it was found on four of the six infected germlings. The other species isolated was F. acuminatum Ell. & Kellerm.

Fusarium was not isolated from root tips of the 13 older seedlings with twisted needles that were sampled. Unidentified species of Penicillium and Trichoderma were commonly isolated.

Douglas-fir Seed Treatment and Germination - Fusarium was found on three of the four seedlots sampled, although infection levels were low (table 3). Fusarium oxysporum was most commonly isolated from seed. The other Fusarium isolated was F. acuminatum; it only occurred on lot A. Surprisingly, seed subjected to the running water rinse (treatment 3) had higher counts of Fusarium than the other treatments. However, samples were too small for statistical analysis and differences may therefore only reflect sampling variability. Treatment with hydrogen peroxide did not eliminate Fusarium on seedcoats or within endosperms. Common saprophytic seed inhabitants including Penicillium spp., Trichoderma spp., Mucor sp. and Aureobasidium pullulans (deB.) Arn. were prevalent.

Table 1.--Douglas-fir seed treatments for selected seedlots from the Plum Creek Nursery.

Treatment no.	Description
1	Seeds placed directly on selective medium (Komada 1975).
2	Seeds soaked in standing water for 24 hours and placed on selective medium.
3	Seeds rinsed under continuous flowing tap water for 48 hours (agitated) and placed on selective medium.
4	Seed surface sterilized (2 hours in 3 percent H_2O_2 - rinsed with distilled water), aseptically dissected to exposure endosperm and inner seedcoat and placed on selective medium.
5	Seed surface sterilized (as in treatment 4) and placed on selective medium.
6	Seed surface sterilized (as in treatment 4) and placed on moistened filter paper (germination test).
7	Seeds rinsed (as in treatment 3) and placed on moistened filter paper (germination test).
8	Seeds soaked (as in treatment 2) and placed on moistened filter paper (germination test).

Table 2.--Results of isolations from Douglas-fir germlings with tap root lesions from the Plum Creek Nursery.[1]

Germling no.	Percent of root system colonized by *Fusarium*[2]	*Fusarium* species[3]
5	23.1	FOXY; FACU
6	6.7	FACU
8	15.4	FOXY
11	6.7	FACU
12	7.7	FOXY
13	6.7	FOXY

[1] *Fusarium* was not recovered from seven of the 13 germlings (numbers 1-4, 7, 9-10).

[2] Average for infected seedlings = 10 percent of the root length colonized by *Fusarium*.

[3] FOXY = *Fusarium oxysporum*; FACU = *Fusarium acuminatum*.

Table 3.--Treatment effects on occurrence of Fusarium and other fungi on Douglas-fir seed from the Plum Creek Nursery.

	Percentage of seed with Fusarium Seedlots				Other fungi[2] Seedlots			
Treatment[1]	A	B	C	D	A	B	C	D
1	0	0	0	0	PEN-TRI	PEN	PEN	PEN-TRI
2	0	0	0	3.3[3]	PEN-TRI	PEN	PEN-TRI	PEN-TRI
3	26.7[4]	0	6.7[3]	3.3[3]	PEN-TRI-	PEN-TRI-	PEN-TRI-	PEN-TRI
4	0	0	0	3.3[3]	PEN-TRI-MUC	PEN-TRI-AUR	PEN-TRI-MUC	PEN-TRI
5	6.7[3]	0	0	3.3[3]	PEN-TRI	TRI-AUR	PEN-TRI	PEN-TRI
All treatments	7.7	0	1.3	2.3				

[1]See table 1 for description of treatments.

[2]PEN = Penicillium spp.; TRI - Trichoderma spp.; MUC = Mucor sp.; AUR = Aureobasidium pullulans

[3]Fusarium oxysporum

[4]Fusarium acuminatum

Treatment effects on seed germination are summarized in table 4. Treatments with hydrogen peroxide decreased early germination (within 7 days) of three of the four seedlots tested. However, after 19 days, germination was generally comparable to the water treatments. Most nongerminated seed had decayed endosperms or were empty; the amount of decayed/empty seed was highest in seedlots A and D.

Table 4.--Effects of treatments on germination of Douglas-fir seed from the Plum Creek Nursery.[1]

Seedlot	Treatments[2] 6	7	8	All
A				
Germinated - 7 days	53	69	68	63.3
Germinated - 19 days	72	78	76	75.3
Nongerminated (19 days) - decayed	24	21	24	23.0
Nongerminated (19 days) - healthy	4	1	0	1.7
B				
Germinated - 7 days	67	85	77	76.5
Germinated - 19 days	91	88	90	89.6
Nongerminated (19 days) - decayed	5	8	7	6.6
Nongerminated (19 days) - healthy	4	4	3	3.8
C				
Germinated - 7 days	55	73	72	66.4
Germinated - 19 days	81	85	78	81.5
Nongerminated (19 days) - decayed	14	9	18	13.7
Nongerminated (19 days) - healthy	5	6	4	4.8
D				
Germinated - 7 days	53	52	27	46.5
Germinated - 19 days	67	65	46	60.2
Nongerminated (19 days) - decayed	31	32	50	37.0
Nongerminated (19 days) - healthy	2	3	4	2.8

[1]Values in table are percentages.

[2]See table 1 for description of treatments.

DISCUSSION

The 1985 Douglas-fir crop at the Plum Creek Nursery had very few disease problems. Early damping-off losses were low and probably maintained at low levels due to timely fungicide applications. Root disease or late damping-off, common in several other nurseries in the northern Rocky Mountains (James 1983a; James 1984b; James 1985b), was not encountered during 1985.

Occurrence of lesions on the taproot of young germlings was not always associated with infection by Fusarium or other pathogenic fungi. Infected germlings appeared healthy; lesions were only found during thinning operations. Because occurrence of Fusarium on asymptomatic seedlings is common (Bloomberg 1966; James 1984b; James 1984c), growers should not necessarily be concerned if the fungus is present on seedlings. Since root disease symptoms

did not develop later during the growing season, the Fusarium present may have been nonpathogenic or incapable of initiating disease because of low inoculum potential or nonsusceptible host material.

Extent of Fusarium on Douglas-fir seed was well within the range found at several other nurseries. For example, levels at the USDA Forest Service Nursery in Coeur d'Alene, Idaho ranged from 2-8 percent (James 1983b); those at the Champion Timberlands and Montana State nurseries in Montana were from 0-13 and 2-4 percent, respectively (James 1984a; James 1984c). Most samples of Plum Creek seedlots yielded infection rates below 7 percent. The one exception (seedlot A) was the result of infection by F. acuminatum, which may have been an unusual situation since none of the other seedlots were infected by this species.

Because of low background infection levels, it was difficult to find significant differences in the amount of Fusarium on seed as influenced by various treatments. Seedcoats commonly contained high populations of Penicillium and Trichoderma, which may have been important in keeping Fusarium levels low. Rinsing seed under continuous running water for 48 hours resulted in more Fusarium being detected on seedcoats. Whether this was because of reduced occurrence of competitive fungi on seedcoats due to the washing was unknown. Since samples were of insufficient magnitude for statistical comparisons, differences may have been due to sampling variability and, therefore, not significant. With the exception of seedlot D, differences in seed germination due to treatments were not evident. For seedlot D, soaking seed in standing water resulted in decreased germination. However, this lot also had a much higher proportion of its seed that did not germinate because of decayed endosperms. Some of this decay may have been due to Fusarium, but other seed colonizers were probably important as well.

Occurrence of F. oxysporum as the major Fusarium species colonizing Douglas-fir germlings and seed confirms previous work (James 1983b; James 1984a; James 1984c). Although this fungus is often associated with conifer seedling diseases, strains of different pathogenic ability usually exist in nature (Gordon 1965; Graham and Linderman 1983; James and Gilligan 1984). Common occurrence of pathogenic strains of the fungus at the Plum Creek Nursery was not evident.

Fusarium acuminatum has previously been associated with containerized ponderosa pine seedling mortality (James 1985b) and shown to be highly pathogenic on several different conifer species (James and Gilligan 1984). Its occurrence on only one of the tested Plum Creek seedlots indicates that it is probably not widespread on Douglas-fir seeds. However, its relative abundance and importance requires further investigation.

In conclusion, investigations of the 1985 crop of containerized Douglas-fir seedlings and selected seed at the Plum Creek Nursery indicate that occurrence of Fusarium as a pathogen was rare. Low inoculum and proper growing regimes which limited fungal buildup and maintained vigorous stock probably accounted for low disease incidence.

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